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14. ABSTRACT:
Ongoing efforts are focused upon generation of KRas mut Smac-/- mouse model of lung cancer since there was a delay in obtaining the smac knockout mice. We were able to demonstrate the feasibility of delivering SBRT to the KRas mutant mouse model of lung cancer as described in the following publication: Du S, Lockamy V, Zhou L, Xue C, LeBlanc J, Glenn S, Shukla G, Yu Y, Dicker AP, Leeper DB, Lu Y, **Lu B.** Stereotactic Body Radiotherapy Delivery in a Genetically Engineered Mouse Model of Lung Cancer. *Int J Radiat Oncol Biol Phys,* 2016 Nov 1;96(3):529-37. We have continued our studies using subcutaneous mouse model of lung cancer by demonstrating that CD8 T lymphocytes and TNFa are necessary to mediate the therapeutic synergism between ablative radiotherapy and a smac mimetic. Since anti-PD1 immunotherapy is shown to be superior to platin-based cytotoxic chemotherapy and change the landscape of lung cancer treatment, we have determined and demonstrated potential synergism from the combination of anti-PD1 therapy and a smac mimetic. These results will be validated in the genetically engineered mouse models once they are available.

15. SUBJECT TERMS

SMAC, apoptosis, lung cancer, immunotherapy, radiotherapy

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PI: Bo Lu
Progress Report: Year One

1. Introduction: Localized advanced and metastatic non-small cell lung cancers (NSCLC) frequently develop therapeutic resistance and recurrences following the standard chemoradiotherapy. No significant progress has been made in the cure rate of lung cancer over the past two decades. Immunotherapy such as PD-1 inhibitors are now approved for the treatment of lung cancer. The integration of immune enhancers into radiotherapy regimens has the potential of rescuing therapeutic immunity against lung cancer cells as recent immunotherapeutic trials utilizing immune checkpoint inhibitors, such as anti-PD-1/PDL-1, have yielded impressive and durable responses among certain lung cancer patients [1-2]. In this case, the release of cancer antigens following radiotherapy may synergize in the induction of cancer cell-specific immune responses, which may impact distant metastases [3]. While confirming that the approach has merit, recent clinical trials have documented that less than 20% of patients with NSCLC respond to PD-1/PDL-1 inhibitors [1-2], highlighting the need for alternative approaches to improve the outcome, particularly of patients who respond poorly to PD1 inhibitors. We have obtained data indicating that the combined use of a SMAC mimetic and radiotherapy could result in therapeutic synergism in a lung cancer model through a CD8- dependent mechanism. We intend to extend this work by determining the role of SMAC in lung cancer progression and its resistance to therapy and determine immunological mechanism by which combining a SMAC mimetic and radiotherapy yields therapeutic synergy and whether this combination yields abscopal effects from radiotherapy.

2. Keywords: smac, lung cancer, immunity, radiotherapy

3. Accomplishments: Progress of this project is outlined below:

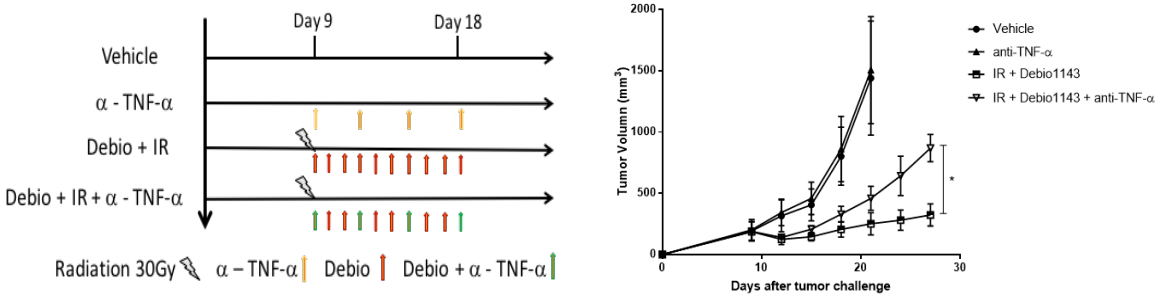
Aim 1: Determine whether the loss of SMAC promotes carcinogenesis and interferes lung cancer therapy: Ongoing efforts are focused upon generation of KRas mut Smac-/- mouse model of lung cancer since there was a delay in obtaining the smac knockout mice.

Aim 2: Determine the abscopal effect and optimize the therapeutic ratio of combining a SMAC mimetics and Stereotactic Body Radiotherapy (SBRT) in mouse models of lung cancer: We were able to demonstrate the feasibility of delivering SBRT to the KRas mutant mouse model of lung cancer as described in the following publication: Du S, Lockamy V, Zhou L, Xue C, LeBlanc J, Glenn S, Shukla G, Yu Y, Dicker AP, Leeper DB, Lu Y, **Lu B**. Stereotactic Body Radiotherapy Delivery in a Genetically Engineered Mouse Model of Lung Cancer. *Int J Radiat Oncol Biol Phys,* 2016 Nov 1;96(3):529-37.

We have continued our studies using subcutaneous mouse model of lung cancer by demonstrating that CD8 T lymphocytes and TNFa are necessary to mediate the therapeutic synergism between ablative radiotherapy and a smac mimetic. Since anti-PD1 immunotherapy is shown to be superior to platin-based cytotoxic chemotherapy and change the landscape of lung cancer treatment, we have determined and demonstrated potential synergism from the combination of anti-PD1 therapy and a smac mimetic. These results will be validated in the genetically engineered mouse models once they are available.

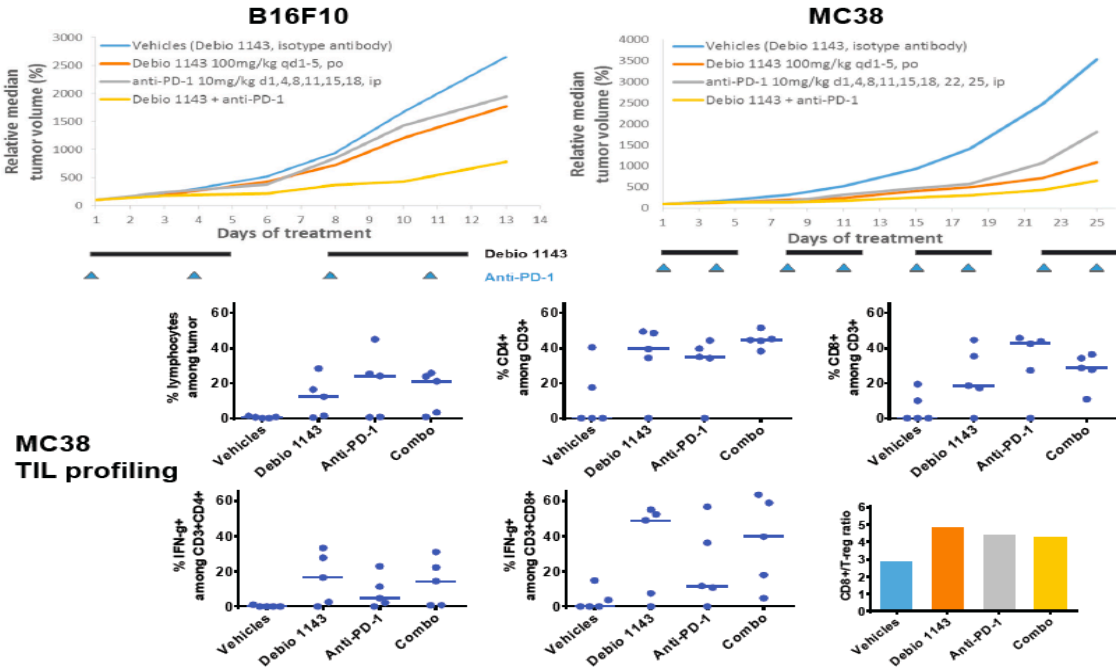
TNF-α is essential for the efficacy of combination treatment

We observed that combining smac mimetic and radiotherapy led to significant reduction of MDSCs in the treated tumors. To characterize the role of TNF-α for the reduction of MDSCs after combination therapy, we performed neutralization experiments in vivo. C57BL/6 mice were inoculated with LLC-OVA cells and tumors allowed to develop; treatment was begun with α-TNF-α antibody given every 3 days beginning on day 9 for four times. When systemic blockade of TNF-α was applied, we observed a reversal of the efficacy of combined Debio 1143, a smac mimetic and radiotherapy ($p<0.05$). To determine whether TNF-α-mediated cytotoxicity was necessary and sufficient to induce cell death and eliminate MDSCs. We examined the changes in percentage of MDSCs after blocking TNF-α in combination therapy. Our results indicate that eliminating TNF-α partially restores the levels of MDSCs to those observed in combination treatment group ($p<0.01$). Taken together, these data suggest that the reduction of MDSCs following combination treatment with Debio 1143 and radiotherapy facilitates tumor regression mediated by TNF-α as shown below.

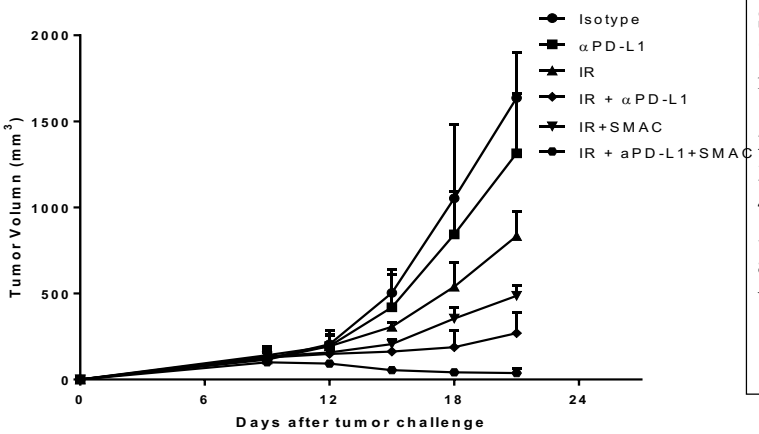


Debio 1143 enhances the efficacy of anti-PD1and increases tumor-infiltrating lymphocytes:

Given that combining immunomodulatory agents can potentially lead to therapeutic synergy, we tested the potential therapeutic synergism between Debio 1143 and anti-PD1 in both the B16 melanoma model and the MC38 colon cancer model. As shown below, there is a clear synergism between the two agents in both tumor models, as measured by tumor volumes. Tumor infiltrating lymphocytes (TILs) were significantly increased in tumors treated with either agent or combined. These TILs demonstrated active IFN-γ-expressing CD4+ or CD8+ phenotype.



Triple combination leads to superior therapeutic efficacy*:* C57Bl/6 mice were inoculated with ovalbumin-expressing Lewis lung carcinoma cells (LLC-OVA). On day 9, as tumors reached a size around 0.5 cm^3, mice were treated with 10Gy and another dose in the following day, and Debio 1143 (100mg/kg) was given on day 9 through day 15. Anti-PDL1 antibody (200ug) was given every 3 days, from day 9 through day 18. As shown in Figure 6, the triple combination resulted in tumor regression while the alternative therapies only attenuated tumor growth.



Triple combination of anti-PDL1, Smac mimetic and radiotherapy. Subcutaneous LLC-OVA tumors (10 mice per group) were treated by:
1). IgG control;
2). Anti-PDL1;
3). Radiotherapy;
4). Radiotherapy and Debio1143;
5). Radiotherapy and Debio1143 and anti-PDL1 antibody. Tumor volumes were measured and plotted over time

Impact: Smac mimetics may enhance immunotherapy as well as radiotherapy

Changes/Problems: Since systemic therapy of lung cancer has evolved from predominant cisplatin chemotherapy to anti-PD1 immunotherapy, we explore the therapeutic synergism and the underlying mechanism from the combination of anti-PD1 and smac mimetics. We have encountered a problem of generating KRas mut Smac-/- mouse model of lung cancer since there was a delay in obtaining the smac knockout mice.